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TRANSLATION NO. 2318

DATE: Feb. 1966

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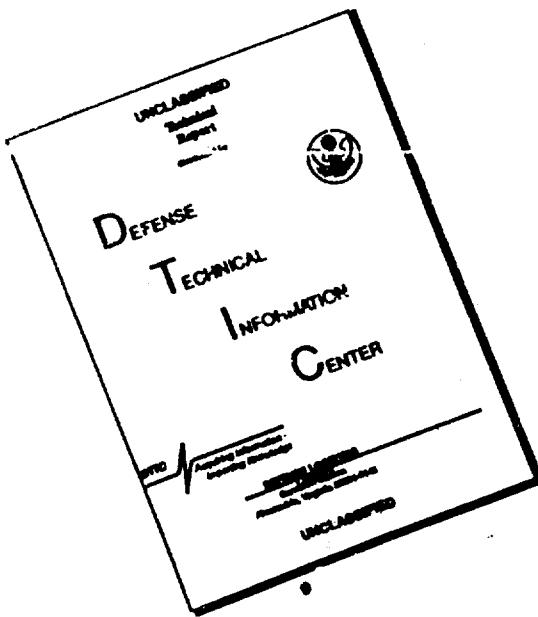
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Gigiena i Sanitaria (Moskva) 27, No. 9, 1962, pp. 111-113

Dissemination of Cl. Botulinum and Cl. Tetani in the Soil of Some Regions in the Armenian SSR

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(Received September 7, 1961)

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On the basis of conducted soil tests and examinations of the gastrointestinal tract contents of fishes, A.T. KRAVCHENKO et al. (1960) and L.M. SHISHULINA (1961) reported a general dissemination of Cl. botulinum throughout the territory of the USSR. The greatest dissemination of the causative agent of botulism was observed in the soil of southern regions of the country (northern CAUCASUS, FERGANA) and in the silt-covered shores of some lakes and river basins (western shores of the CASPIAN SEA, AZOV SEA and DON River delta). Thus, in the regions that have a high percentage of the soil infection by botulism bacterium there is a potential danger of flareups as well as of sporadic cases of botulism. Our attention was drawn to the fact that in recent years botulism cases were reported in the Armenian SSR that resulted from eating canned (common) purslane; its production began on a small scale at the end of 1949. Then, as the canning advanced to a large scale output in 1953, first instances of poisonings appeared. The cause was the inferior condition of canned food due to incomplete removal of the soil and sand particles from the original staples (A.V.ZAKARYAN).

et al., 1960). Thus, it was expedient to ascertain a dissemination degree of *Clostridium botulinum* in the soil of various regions in the Armenian SSR.

We found no indicants in the literature as to any dissemination of *Clostridium tetani* on the territory of this republic. Yet, on the basis of high incidence of tetanus in the Armenian SSR (1.4 cases per 100,000 population), K.I. MATVEEV (1960) included this republic in the first zone, i.e. in the zone with the highest coefficient of the disease rate (1.3 to 5.7 per 100,000 population). These findings provided a basis for assumption that there is a considerable dissemination of *Clostridium tetani* in the soil and in the surrounding media of the Armenian SSR. In order to clarify the conditions, we conducted soil tests in some regions of the republic. The investigation technique has been described in reports of A.T. KRAVCHENKO, L.M. SHISHULINA, G.P. GALKINA and L.V. GORETSKA (1960). Each sample of the soil was suspended in 1% peptone water. One part of the suspension was inoculated on the KITT-TAROCCI culture medium, following a prior heating in water bath at 80° for 30 minutes and after cultivation period at 34°; the other part was inoculated without a prior heating but following incubation at 25°. The activity of each inoculation was tested on the 5th and 14th day of its growth by means of intraperitoneal administration to 2 white mice a dose of 0.5 ml of the culture fluid in a concentration of 1:2.

The determination of all active specimens was carried out by the neutralization method of the toxin by using polyvalent anti-botulism and antitetanic serums and, then, by monovalent antitoxins serums.

Clostridium botulinum was detected in 9 specimens (14.3%) and *Clostridium tetani* in 18 specimens (28.5%). We observed a diversified dissemination of the causative agent of botulism and that of tetanus in the soil of various examined localities. We detected *Clostridium tetani* in 7 out of 8 specimens in the soil used for vineyards, but none in the soil taken from the RAZDAN River shore; then, in the vicinity of the SEVAN Lake we detected tetanic rod in 2 specimens of the soil out 10 examined samples. The degree of the soil infection by tetanus was approximately the same in other localities. Among the examined sections the highest dissemination degree of the causative agent of botulism was found in the soil along the shore of the GEDAR River, in the vicinity of the collective farm's market place of YEREVAN, where, out of 9 examined specimens, we detected 5 positive samples. The shore of the GEDAR River has been polluted by waste matter and this, perhaps, contributed to the preservation and breeding of *Clostridium botulinum*. In contrast with this, we detected only 2 samples containing *Clostridium botulinum* out of 14 examined soil specimens taken from the shores locality of the mountain river RAZDAN where it passes close to the outskirts of the town. Here, *Clostridium botulinum* was found in the soil taken from the mountain slope located about 100 m from the SEVAN Lake.

Our findings revealed that in the soil of the Armenian SSR can be found the causative agent of botulism of the types A, B, C and E, also that most specimens contained *Clostridium botulinum* type A (5 samples contained *Clostridium botulinum* type A, 2 samples - type B, 1 sample - types A, B, C and 1 sample - type E). We stated in previous reports that, comparing the dissemination of *Clostridium botulinum* in the

soil throughout the Soviet Union and in individual regions, type B is considered predominant. Yet, the difference in dissemination of various types of the causative agent of botulism speaks for itself, namely that each region has its own peculiarity in dissemination of *Clostridium botulinum* in the soil.

We should also add that most specimens containing *Clostridium botulinum* were not heated prior to inoculation, but were allowed to incubate at 25° (see Table 1). We found that the same samples used for in-

Table 1

Dissemination of *Clostridium Botulinum* Contingent upon the Method of Specimens' Processing

Cultivation temperature	Processing of material prior to inoculation	Quantity of specimens containing <i>Clostridium botulinum</i>			
		A	B	AC	B
25	Without heating	5 ¹⁾	3	--	1
34	Heating at 80° for 30 minutes	1	-	1 ²⁾	-

1) - One sample contained *Clostridium botulinum* in a preheated and in not heated suspension of the soil.

2) - *Clostridium botulinum* type B was detected in this not heated specimen cultivated at 25°.

oculation after heating became inactive, with the exception of one specimen which produced a "nonspecific" activity, namely the progress of the disease ran a nontypical course in white mice with regard to the botulism intoxication and negative reaction following the neutralization of toxin by polyvalent antitoxin serum. Thus, for more specific findings on dissemination of *Clostridium botulinum* in the examined material we recommend that inoculations should include heated as

well as not heated material.

The absence of Cl. botulinum in inoculations of samples heated and then cultivated at 34° can, perhaps, be explained either by the thermolability of spores, or by their negligible quantities among the available vegetative ^{microbial} forms. The latter assumption tends to indicate a breeding of Cl. botulinum in the soil.

Data obtained by us and the presence of botulism cases that resulted from eating canned purslane confirm again the necessity of taking into account the semination of Cl. botulinum in moist grounds and in their environments; this should be particularly considered at the time when a new cannery is organized, or new canned foods are processed.

The high percentage of dissemination of Cl. tetani in the soil confirms the necessity of carrying out preventive measures proposed by K.I. MATVEEV.

Conclusions

1. We detected Cl. botulinum in 9 specimens (14.3%) and Cl. tetani in 18 specimens (28.5%) after examination of 63 samples of soil.
2. The soil in the Armenian SSR contains Cl. botulinum of the A, B, C and E types. The highest dissemination is that of Cl. botulinum type A. Out of 9 specimens containing Cl. botulinum, 5 were obtained in the vicinity of the collective farm's market place (GEDAR River shore) of YEREVAN.
3. In most cases Cl. botulinum was detected after inoculations of soil samples not heated previously; perhaps, this can be explained by the thermolability of spores, or by the availability of vegetative

microorganic forms.

4. Enclosures of soil used for a vineyard and orchard revealed the highest dissemination of Cl. tetani.

Literature Cited

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